Reproductive Biology and Gametophyte Morphology of New World Populations of Acrostichum aureum

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The majority of homosporous ferns are characterized by a life-cycle which permits the production of a genetically homozygous zygote following self-fertilization of a single gametophyte (intragametophytic selfing). This homozygosity leads to the expression of recessive deleterious or lethal genes (genetic load, as defined here) present in the genotype, unless this expression is buffered by the polyploid system. Sporophytes expressing such genes will be eliminated rapidly and the spore genotypes produced individually by the remaining viable sporophytes will be genetically uniform, barring mutation and meiotic irregularities (e.g., homeologous pairing; Klekowski, 1979). In species which regularly undergo selfing, genetic load will be absent or will be expressed at low levels (Klekowski, 1979). Thus, analysis of genotypes for genetic load allows for an estimate of the genetic variability in a population.

The fern life-cycle also permits reproduction which is genetically analogous to inbreeding and outbreeding in angiosperms, the latter facilitating the storage of recessive deleterious and lethal genes (Wallace, 1970). Although none of the above patterns of reproduction are mutually exclusive, work of the past decade has led to the hypothesis that specific morphological and developmental features of the gametophyte generation will increase the probability of selfing or crossing (intergametophytic mating) and that these probabilities can be correlated with estimates of heterozygosity in the form of genetic load (Lloyd, 1974). However, more recent work with *Ceratopteris* (Lloyd & Warne, 1978) and *Acrostichum* (Lloyd & Gregg, 1975) suggests that the past hypotheses are insufficient to explain the genetic diversity expressed in these species and that other factors are involved. This paper summarizes our most recent work on the gametophyte morphology, reproductive biology, and genetic diversity in a number of populations of *Acrostichum aureum* distributed from Florida to the northern coast of South America and attempts to circumscribe the current problems in this field.

The genus *Acrostichum* consists of at least three species: *A. danaeifolium* Langsd. & Fisch., a New World endemic which is widely distributed in fresh water and slightly saline swamps (Adams & Tomlinson, 1979); *A. aureum* L., circumtropical in distribution and usually most abundant in mangrove habitats where it can withstand partial tidal immersion (Holttum, 1954; Small, 1938); and *A. speciosum* Willd., a species of tropical Asia and Australia which is abundant in mangroves through Malaya in areas frequently inundated by tides (Holttum, 1954). These types of habitats are extreme; few species of plants have evolved the necessary physiological and morphological features to successfully colonize them. Previous work on the gametophyte generation in *Acrostichum* includes mor-

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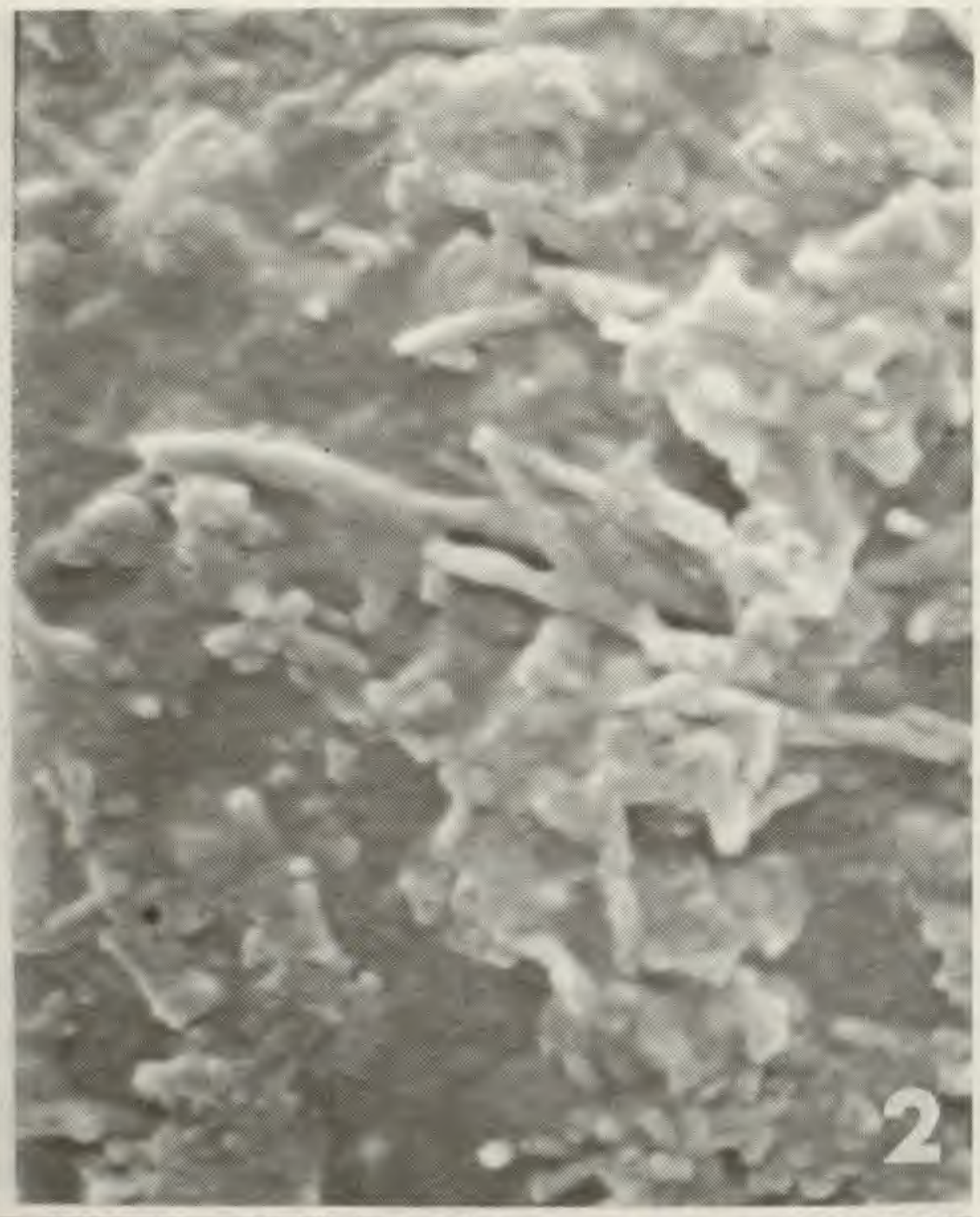
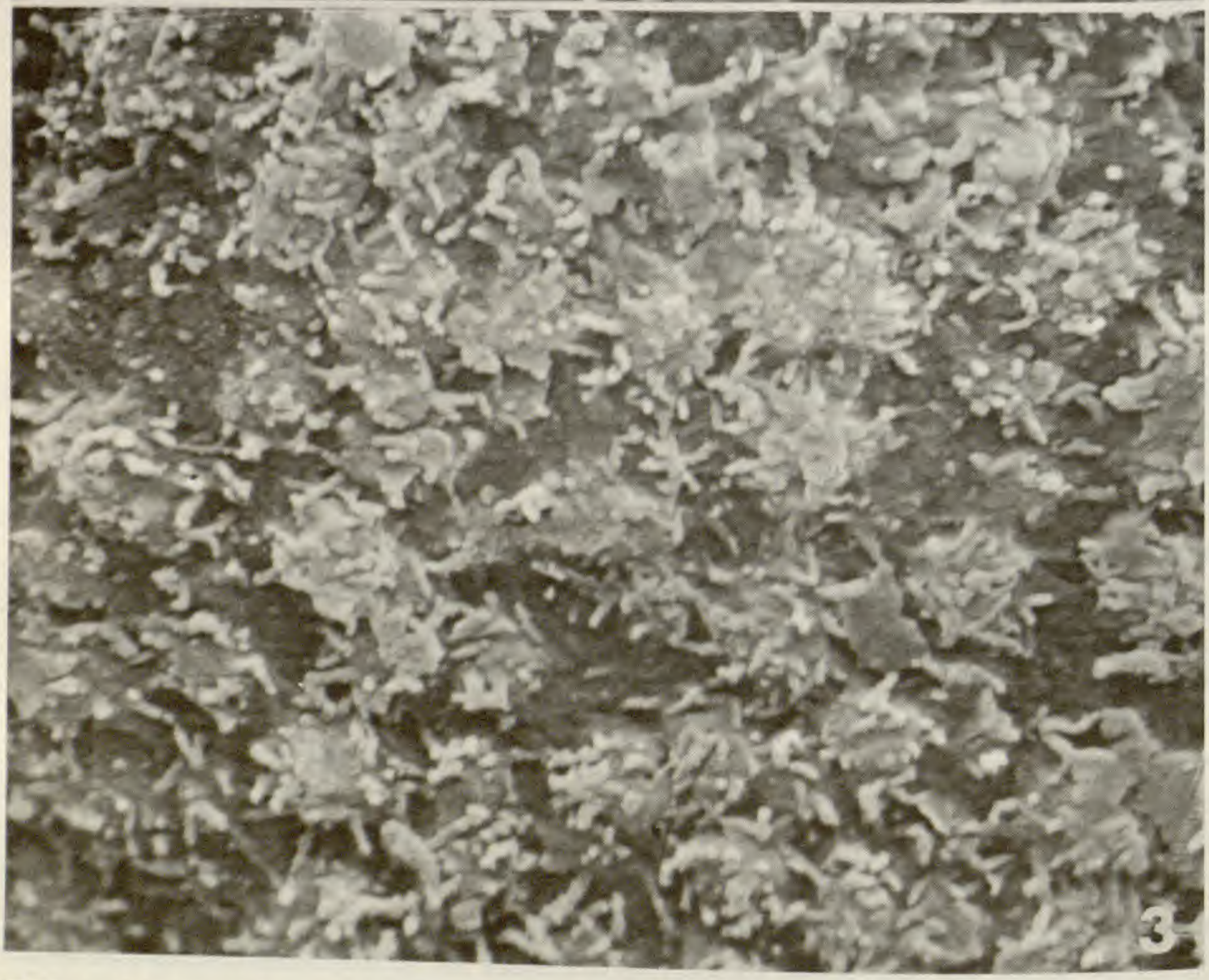




FIGS. 1–3. Spores of *A. aureum*. FIG. 1. Papua, *Brass 518* (UC), × 1000. FIG. 2. Florida, *Curtiss 5463* (UC), × 10000. FIG. 3. Papua, *Brass 518* (UC), × 8000.

phological studies of *A. speciosum* by Stokey & Atkinson (1952) and a study of the morphology and reproductive biology of Mexican populations of *A. danaeifolium* by Lloyd & Gregg (1975).

MATERIALS AND METHODS

Spores of *A. aureum* were collected from 39 plants from eight populations as follows:

Culture No. *146:* 1.1 mi W of U. S. Highway 41 on State Highway 92, Collier Co., Florida. *148:* 2.1 mi W of Westlake on State Highway 27, Everglades National Park, Dade Co., Florida. *150:* 30.5 mi SW of entrance station on State Highway 27 at road to Westlake, Everglades National Park, Monroe Co., Florida. *190:* 0.25 mi E of Negril on road to Savana la Mar, Westmoreland Parish, Jamaica. *191:* Mile post 57, 57 mi E of Georgetown on Public Road East, Guyana. *192:* 8 km N of Governor's Palace, Parimaribo, near end of road to Leonsburg, Suriname. *193:* 0.2 mi from road to Colón on road to Coco Solo, Canal Zone, Panama. *194:* Lowland area near Pacific Ocean at N end of the Bridge of the Americas, Canal Zone, Panama.

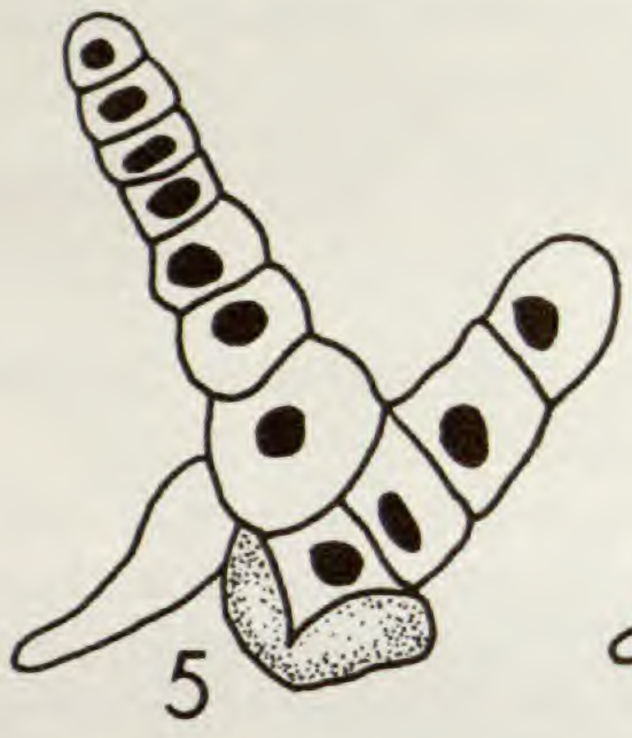
Spores were sown and gametophytes grown aseptically on sterile inorganic nutrient medium solidified with 1% agar (for composition see Klekowski, 1969) in 100 × 15 mm petri dishes. Gametophytes were grown under continuous illumination by fluorescent and incandescent lamps at an intensity of 210 to 290 ft-c at temperatures of 19–24° C. Prothallial morphology was studied using living material as well as that mounted in Hoyer's medium mixed with acetocarmine. Spore sizes were determined by mounting spores in diaphane and calculating their equatorial diameters with a calibrated ocular micrometer. Other methods utilized in specific experiments are described below.

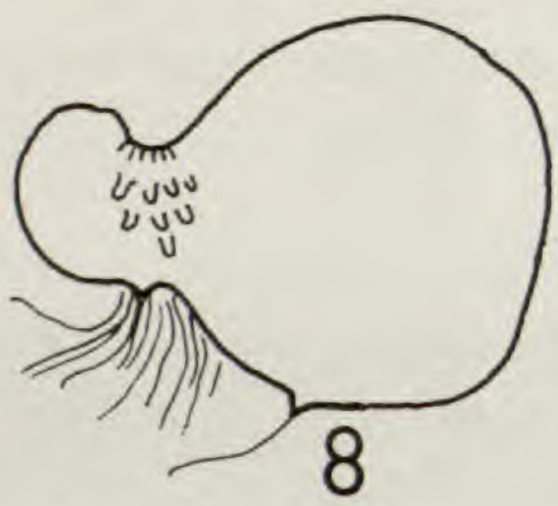
Spores observed by the scanning electron microscope were dry-mounted on double-stick tape, coated with gold ca. 10 nm thick, and observed at 20 kv accelerating voltage with a Hitachi HHS-2R scanning electron microscope.

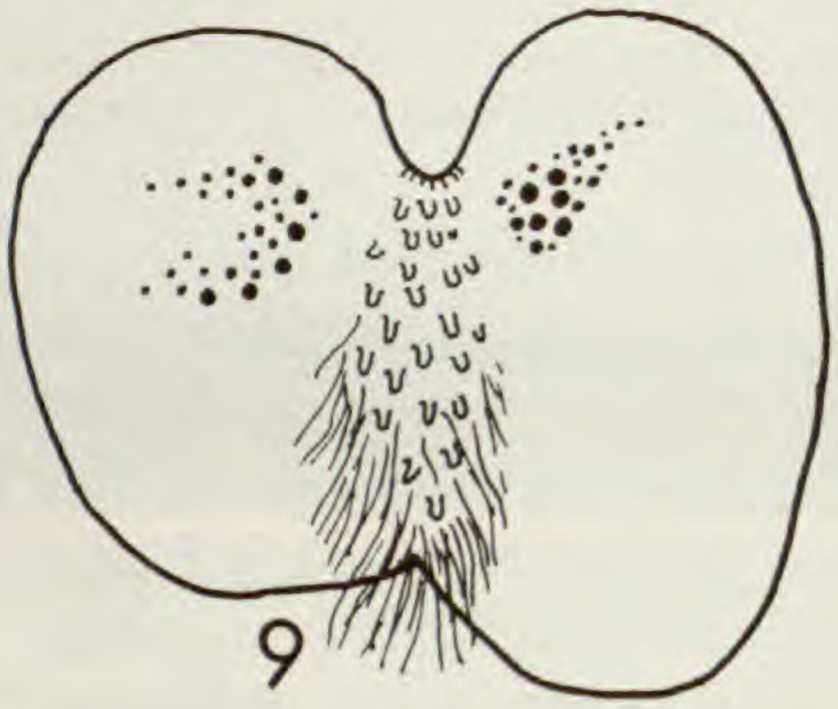
RESULTS

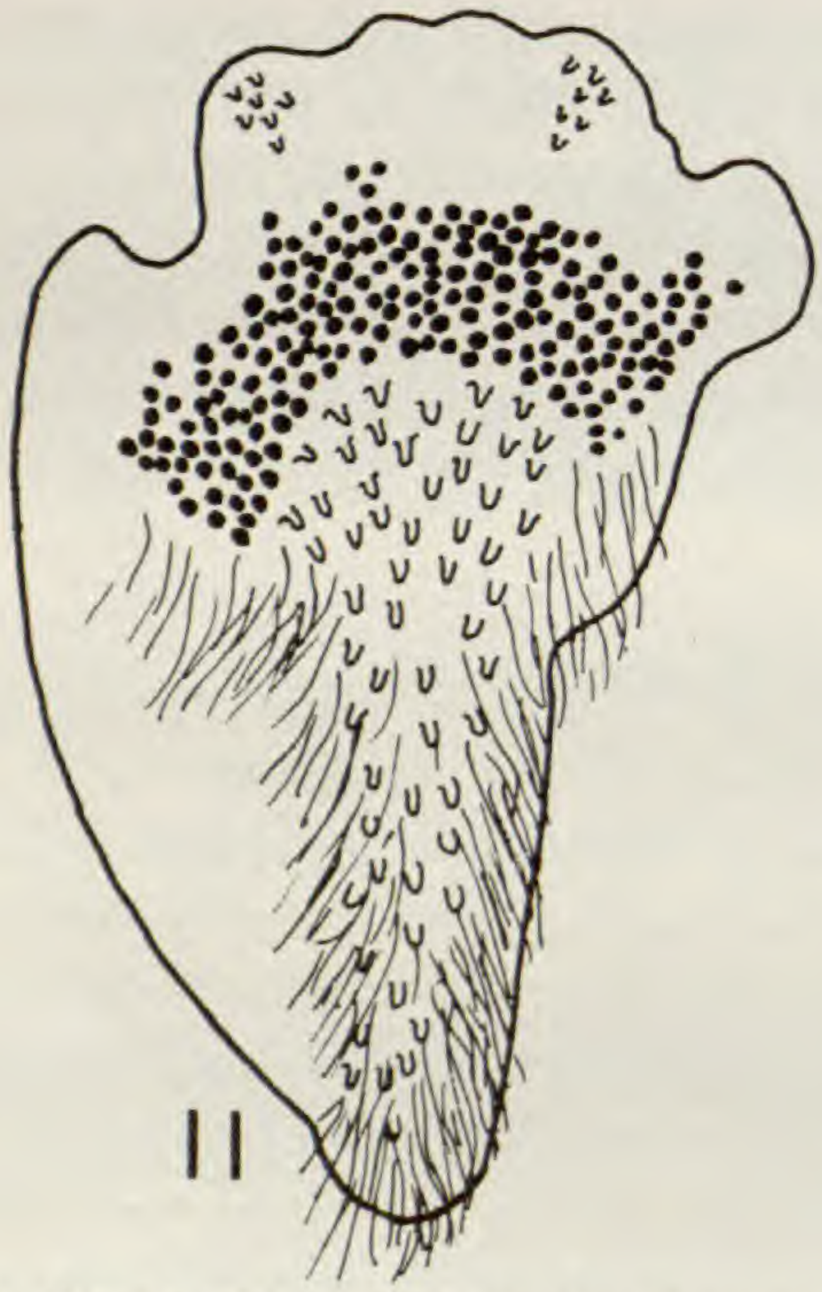
Spores of *A. aureum* are tetrahedral and are (37)45–72 (mean ± s.d. = 56.7 ± 4.58) μm in diameter. The spore surface is minutely tuberculate (*Fig. 1*). The tubercle-like structures on the surface bear varying numbers of projecting papillae. Spores from plants from Papua (*Figs. 1, 3*) and Fiji exhibit numerous but somewhat irregularly shaped and oriented papillae. Spores from Florida plants (*Fig. 2*) and other Fijian plants appear to have more numerous papillae as well as other types of superficial deposits. In contrast, spores examined from plants from Australia and Trinidad are tuberculate but appear either to lack papillae or to have thickened superficial deposits which more or less obscure their presence. Spores examined of *A. speciosum* from Papua exhibit surface features highly similar to those from Florida plants of *A. aureum*.

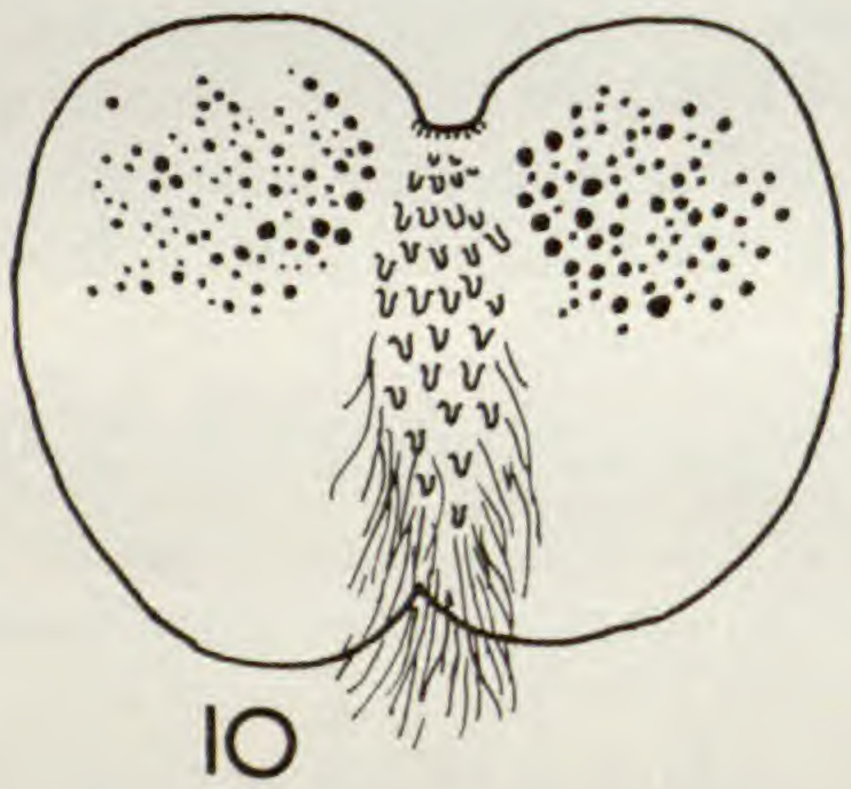
Spore germination is usually initiated by the emergence of a rhizoid five days following sowing. Gametophytes produce a one-dimensional filament up to 10 cells in length before initiation of two-dimensional growth (*Fig. 4*). In some instances, cells near the base of the filament will divide, producing a second one-dimensional filament (*Fig. 5*).











FIGS. 4–11. Stages in gametophyte development and sexually mature gametophytes ot *A. aureum*. FIG. 4. One-dimensional filament with initiation of two-dimensional growth in basal cell, 185 μm long, 10 days after sowing. FIG. 5. Branched gametophyte with two filaments, 196 μm long, 10 days after sowing. FIG. 6. Two-dimensional filament with two-ranked cells, 208 μm long, 10 days after sowing. FIG. 7. Male prothallus with single antheridium, 1.3 mm long, 29 days after sowing. FIG. 8. Female prothallus with asymmetrical wings, 1.5 mm long, 29 days after sowing. FIG. 9. Young hermaphroditic gametophyte with numerous immature antheridial initials and few mature antheridia, 3.4 mm long, 51 days after sowing. FIG. 10. Young hermaphroditic prothallus with numerous gametangia, 4.0 mm long, 51 days after sowing. FIG. 11. Mature gametophyte showing sequential pattern of archegonial-antheridial-archegonial production and branched meristem, 7.5 mm long, 96 days after sowing.

Two-dimensional growth is initiated by a longitudinal division in a central or more basal cell of the one-dimensional filament. Further longitudinal divisions in the filament may follow one or more patterns: cells throughout the filament, except for the terminal and basal cells, may divide longitudinally and produce a two-dimensional filament two cells wide (*Fig. 6*); less frequently, central cells of the 1-dimensional filament may divide sequentially, producing an area up to four or more cells in width before further divisions occur in the more basal and terminal cells of the filament. In both pathways, 2-dimensional growth ultimately results in a broadly linear or spatulate gametophyte four to six cells wide. Further divisions of cells along one of the lateral margins of these prothalli will produce a lateral meristematic region located near the basal region of the gametophyte. Subsequent growth produces an asymmetrical ovate prothallus with different sized wings (*Fig. 8*). In older gametophytes, growth frequently produces more or less symmetrical wing tissue on both sides of the lateral meristem, resulting in a mature prothallus which appears to have an apical meristematic notch (*Figs. 9* and *10*)). This notch area remains shallow in most prothalli observed; in some, however, the meristem exceeds the wing tissue and no notch is evident. In some older gametophytes, the meristematic region becomes quite broad, and, rarely, may divide into two separate regions (*Fig. 11*) with non-meristematic tissue between.

Gametangia initiation in culture was rapid. All cultures, except 193-K[1], exhibited a female to hermaphroditic gametangial sequence of development (*Fig. 8*) with the exception of occasional gametophytes which precociously initiated antheridia (*Fig. 7*). Of the 20 cultures studied in detail, seven produced some male gametophytes, but the percentage of such gametophytes in culture (except 193-K) was less than 6.0 (*Tables 1* and *2*). In all cases these prothalli rapidly became hermaphroditic.

The length of the unisexual female gametophytic stage varied from culture to culture. In one culture (190-D), hermaphroditic prothalli were produced simultaneously with female prothalli. In other cultures (150-I, 193-E, 193-L), gametophytes remained unisexual and female throughout the culture period. In the remaining cultures, hermaphroditic prothalli were produced (2)6–28 (mean ± s.d. = 15.6 ± 5.8) days following appearance of female prothalli (*Table 1*).

Gametangial sequences of individual gametophytes are diverse, but the vast majority of gametophytes exhibited a female to hermaphroditic sequence. Archegonia were initiated on the cushion immediately behind the young lateral meristem and were produced continuously until the gametophytes were fully cordate with a pronounced elongate cushion with numerous senescent gametangia (*Fig. 8*). Antheridia were initiated on wing tissue near the apical notch, initially along the margin of the cushion and later outward toward the wing margins (*Figs. 9* and *10*). Fully mature hermaphroditic gametophytes exhibited antheridia covering both wing surfaces near the apical region of the prothallus (*Fig. 10*). Antheridia have not been observed in the distal portions of the prothallus. In culture 190-A, sam-

[1]Here and elsewhere in this paper the letter following the culture number designates a gametophyte population originating from a specific sporophyte.

ples of gametophytes 46 days after sowing indicated that some of them had produced up to 90 antheridial initials. These prothalli exhibited up to 28 or more senescent, 8 mature, and 50 immature archegonia. Six days later (52 days following sowing), fully mature hermaphroditic prothalli were present, exhibiting up to 60 senescent, 10 mature, and 35 immature archegonia, and over 325 antheridia per wing of which about 12% contained mature spermatozoids.

Gametophytes which were initially male produced only one or two antheridia prior to the initiation of archegonia. In most cases, following maturation and dehiscence of these antheridia, gametophytes became functionally unisexual and female and their subsequent ontogeny paralleled that described above.

TABLE 1. DAYS FROM SOWING TO APPEARANCE OF GAMETOPHYTE-TYPES IN CULTURES OF *A. AUREUM*.

Culture number	*Density/cm²*	*Days to appearance of* Male	Female	Hermaphroditic
146-C	27.7	28	21	38
146-G	6.5	35	29	(35)50
146-L	6.9		35	50
146-M	21.3		28	46
148-B	27.7		28	44
150-A	18.0	51	33	(35)56
150-B	29.7		33	44
150-C	28.2		33	56
150-E	3.9		35	50
150-I	8.1		33	–[1]
150-K	14.1		33	44
190-A	6.1		37	49
190-C	5.2		33	49
190-D	12.6	37	33	(33)46
190-F	31.5	59	33	53
193-A	17.6		28	56
193-B	98.3	44	32	–[2]
193-E	45.7		29	–[3]
193-K	79.1	29	29	39
193-L	1.5		29	–[3]

[1]sampled up to 76 days
[2]sampled up to 85 days
[3]sampled up to 90 days

Some of the gametophytes expressed sequential patterns of functional unisexuality. In 146-C, about 15% of the sampled gametophytes expressed a sequence of archegonial initiation, maturation, and senescence, followed by initiation and maturation of antheridia. Other gametophytes appear to have gone through functional stages in sexual ontogeny from archegoniate to hermaphroditic to antheridiate to archegoniate. This sequence was noted in several older prothalli in 193-A (*Fig. 11*) and in one gametophyte of 150-K. In gametophytes producing proliferations near the base, such as those with two 1-dimensional filaments arising from the single basal cell, some of the proliferations were covered with antheridia, whereas others were only archegoniate.

Culture 193-K was unique among those studied in its expression of large numbers of male prothalli (*Table 2*). The initial ratio of male to female prothalli, excluding asexual prothalli, was 1:1. As the culture developed, male gameto-

phytes increased in frequency to a 3:1 ratio; this; in turn, was followed by an increase in both female and hermaphroditic gametophytes. It is of interest to note that at the end of the observation period, there was a 1.1:1 ratio of male and hermaphroditic prothalli to female prothalli. Male gametophytes in this culture frequently were highly elongate and irregularly formed. Many of them initiated antheridia in the early ontogenetic stages following the attainment of a 2-dimensional morphology. In contrast, female prothalli were larger and appeared to be similar in all respects to the female prothalli of the other cultures.

TABLE 2. SEXUAL ONTOGENY IN AGAR CULTURES OF *A. AUREUM*.

	Sexual expression (%)			
Days from sowing	*Neuter*	*Male*	*Female*	*Hermaphroditic*
	Culture No. 146-C			
21	97.5		2.5	
28	48.6	5.4	45.9	
35	26.4		74.6	
38	8.7		78.3	13.0
44	10.5	2.6	68.4	18.0
47	20.0	5.7	40.0	34.2
	Culture No. 190-A			
33	100.0			
37	64.7		35.3	
43	22.2		77.8	
49	29.4		47.1	23.5
52	28.6		31.4	40.0
55	11.1		40.7	48.1
58	6.7		33.3	60.1
76			14.3	85.7
87				100.0
	Culture No. 193-K			
30–33	38.3	29.8	31.9	
39–45	21.7	56.7	19.1	2.5
48–54	15.9	32.9	50.0	1.2
63		24.4	46.3	29.3
	Culture No. 193-L			
30	71.4		28.6	
40	58.3		41.7	
42	38.5		61.5	
48			100.0	
90			100.0	

Parameters of gametophyte morphology and ontogeny discussed above suggest that intergametophytic mating should be prevalent in gametophyte populations of *Acrostichum aureum*. Specific factors exhibited by the gametophyte generation which increase the probability for intergametophyte mating include the female to hermaphroditic gametangial sequence in most gametophytes studied, the dioecious condition expressed in some populations, and the sequential functional unisexuality expressed in some gametophytes of some populations. Additional sup-

port for this assessment comes from observations on sporophyte production in composite cultures and in timing of the appearance of sporophytes in isolate vs. composite cultures. For example, in culture 146-M, after 83 days 90.9% of the prothalli were unisexual female and 9.1% were hermaphroditic. Examination of sampled prothalli indicated that all of the unisexual female gametophytes had produced one or two young embryos, which would only be possible through intergametophytic mating. In addition, in nearly all cultures sampled sporophyte production in composite culture occurred between 17 and 31 days earlier than in isolate culture. As the gametangial sequence is from female to hermaphroditic in these prothalli, in composite cultures spermatozoids produced by the early hermaphroditic gametophytes will fertilize many of the unisexual female prothalli. In contrast, in isolate culture, each of the gametophytes must become hermaphroditic prior to sporophyte production. This sequence of events has been well documented in studies on *Ceratopteris* by Klekowski (1970a).

TABLE 3. FREQUENCY OF DELETERIOUS SPOROPHYTIC GENOTYPES IN INTRAGAMETOPHYTICALLY SELFED, ISOLATED HERMAPHRODITIC GAMETOPHYTES OF *A. AUREUM*.

Culture number	*Number tested prothalli*	*No. (%) normal*	*No. (%) zygotic lethals*	*No. (%) embryonic lethals*	*No. (%) late sporophytic lethals*	*No. (%) leaky lethals*
146-N	19	18(94.7)	1(5.3)			
148-B	20	19(95.0)	1(5.0)			
150-A	20	19(95.0)	1(5.0)			
150-B	20	17(85.0)		1(5.0)		2(10.0)
150-I	18	17(94.4)	1(5.6)			
190-F	19	15(78.9)	3(15.8)	1(5.3)		
191-B	19	17(89.5)	1(5.2)		1(5.2)	
192-A	40	39(97.5)	1(2.5)			
193-C	20	17(85.0)	1(5.0)			2(10.0)
193-E	20	11(55.0)	9(45.0)			
193-F	8	7(87.5)	1(12.5)			
193-M	20	19(95.0)				1(5.0)
All others:[1]	511	511(100.0)				
Totals:	754	726(96.3)	20(2.65)	2(0.26)	1(0.13)	5(0.66)

[1]Includes 27 cultures: 146-C, 146-G, 146-I, 146-J, 146-L, 146-M, 146-O, 150-C, 150-E, 150-H, 150-K, 190-A, 190-C, 190-D, 190-I, 191-D, 192-C, 193-A, 193-B, 193-D, 193-G, 193-H, 193-I, 193-K, 193-L, 193-N, 194.

Additional information relative to reproductive biology can be obtained by analyzing frequency of deleterious or lethal genes (genetic load) expressed in sporophytes (Klekowski, 1979). To analyze genetic load in *A. aureum*, from 20 to 40 gametophytes per sporophyte (= a gametophyte family), prior to the attainment of sexual maturity, were selected at random from stock cultures and individually isolated in 60 × 20 mm petri dishes containing nutrient agar. Following growth, cultures were watered twice weekly to facilitate fertilization, and the resultant sporophytes were allowed to develop to the third frond stage. Results of these studies are given in *Table 3*.

Genetic load was determined as the percentage of the hermaphroditic gametophytes per gametophyte family which failed to yield normal sporophytes. Families exhibiting genetic load in a portion of the gametophytes tested are considered to be expressing heterozygosity in their gametophytic genotypes. Expressions of genetic load were in the form of zygotic lethals (in 2.65% of the 754 gametophytes tested), embryonic lethals (in 0.26%), late sporophytic lethals (in 0.13%) and leaky lethals (in 0.66%) (see Klekowski, 1970b, 1979, for complete discussion of these genetic expressions). It is significant to note that 96.3% of the tested prothalli did not exhibit any deleterious or lethal genotypes. Of the 39 sporophytes tested, 27 (69.2%) were devoid of genetic load (*Table 4*). In the 12 sporophytes expressing load, it varied from 5.0% (cultures 148-B, 150-A, 193-M) to 45.0% (culture 193-E). The mean genetic load for all plants tested was 3.7%.

TABLE 4. GENETIC LOAD IN *A. AUREUM* RELATIVE TO SIZE AND LOCATION OF THE POPULATION.

Population culture number	*Location*	*Size (est. no. plants)*	*Range ($\bar{x}$) % genetic load*	*No. plants tested*	*No. (%) plants with genetic load*
193	Panama	3000	0–45.0 (5.96)	13	4(30.8)
190	Jamaica	1000	0–21.1 (4.22)	5	1(20.0)
191	Guyana	400	0–10.4 (5.2)	2	1(50.0)
150	Florida	75–100	0–15.0 (3.65)	7	3(42.8)
146	Florida	25–50	0–5.3 (0.66)	8	1(12.5)
148	Florida	20	5.0	1	1(100.0)
194	Panama	15	0	1	0(00.0)
192	Surinam	8	0–2.5 (1.25)	2	1(50.0)
Total:			0–45.0 (3.24)	39	12(30.8)

Although the number of plants tested from each population is insufficient for statistical comparison, it is of interest to note that those sporophytes exhibiting the higher genetic load values are found in the larger populations and that the small populations (with 50 or fewer individuals) have very low levels of recessive deleterious or lethal genes (*Table 4*).

Leaky lethal expression (Klekowski, 1970b) was noted in three gametophyte families. In 150-B, normal sporophytes appeared on the two prothalli 165 days after sowing and 38 days following normal sporophyte production of the remaining prothalli tested. Each of these two prothalli exhibited several abortive embryos, indicating that previous selfing had occurred involving lethal genetic combinations. In 193-M, the first sporophyte which appeared was abnormal and exhibited a long, cylindrical, tubular growth with ruffled margins. Subsequent sporophytes from other fertilizations produced normal fronds.

Apomictic proliferations were noted on only one gametophyte in 147-L. Ninety days after sowing, this prothallus proliferated a blade of tissue bearing rhizoids on one margin and small epidermal cells similar to those found on young sporophytes. Irregularly organized vascular tissue was present near the base of this blade, but no roots or stomata were noted.

DISCUSSION

Gametophyte Morphology.—The gametophyte morphology and ontogeny of *A. aureum* is remarkably similar to that of *A. danaeifolium* (Lloyd & Gregg, 1975) and agrees in most respects with that of *A. speciosum* Willd. (Stokey & Atkinson, 1952). Spores of *A. aureum* are almost identical in size and shape to those of *A. danaeifolium*; however, there are minute differences in spore surface markings, especially the more pronounced tuberculate pattern exhibited by *A. aureum*. Other gametophyte features which are qualitatively similar between the two species are formation of the 1-dimensional filament, the lateral meristematic region of the 2-dimensional prothallus, the relatively shallow apical notch region (however, protruding beyond the wing tissue in some prothalli of *A. aureum*), the female to hermaphroditic gametangial sequence, and the sexual expression in gametophyte families grown in composite culture.

The major difference between gametophytes of the two species is the distribution of antheridia, which are mostly restricted to the apical wing and meristem region of *A. aureum*, but also are found in more basal regions along the cushion margins and among the rhizoids in *A. danaeifolium*. In addition, the sequential production of archegonia-antheridia-archegonia in some prothalli of *A. aureum* is unknown in the other species.

It is apparent from both sporophyte and gametophyte studies that these two species are closely related. Further evidence in support of this is their ability to freely hybridize in culture and to produce normal viable F_1 sporophytes, although these sporophytes have not yet been grown to maturity to measure chromosome homology (Lloyd, unpubl.).

Reproductive Biology.—Sex ontogeny in most cultures of *Acrostichum aureum* sampled in this study is female to hermaphroditic or initially dioecious. The length of the unisexual stage prior to the attainment of bisexuality is sufficient to facilitate intergametophytic mating. The facility for such mating is also evidenced in culture by the rapidity of embryo formation in unisexual prothalli following the initiation of antheridia on just one gametophyte in a composite culture. Thus, the gametophytic developmental pathway must be considered as one which has a higher probability of intergametophytic mating than of intragametophytic selfing. However, correlative heterozygosity in the form of genetic load is insufficient in naturally occurring sporophytes to suggest that outbreeding is a normal occurrence. For example, of the tested plants 69% exhibited no heterozygosity for recessive deleterious genes and 15% exhibited such genes in less than 6% of the genotypes sampled. As intergametophytic mating is strongly suggested by the culture experiments, if the assumption is made that these plants are genetically homozygous due to the lack of genetic load expression, other factors must be superimposed upon the hypothesized mating system which are more significant in determining the genetic composition of the populations as a whole.

First and foremost, the culture methodology as used in these experiments may be insufficient to document with accuracy the gametangial sequences as they are realized in nature. In parallel experiments on *A. danaeifolium*, gametophytes

grown on soil exhibit greater antheridial production (Lloyd & Gregg, 1975). Although some of these gametophytes undergo a male to hermaphroditic gametangial ontogeny, dioecism in cultures was still highly prevalent, suggesting that soil grown gametophyte populations in nature would have higher probabilities of intergametophytic mating. As gametophytic ontogenies on agar cultures of *A. aureum* and *A. danaeifolium* are highly similar, it is reasonable to assume that the gametophytes of *A. aureum* would present similar responses to soil culture. However, the habitat of *A. aureum* is at least partially inundated by tides, suggesting that the soil component for gametophyte populations will contain higher levels of salts. Brief experiments by Stokey & Atkinson (1952) using dilute sea water as part of the culture medium induced restricted growth of gametophytes of *A. speciosum*. This type of reduced growth under less than optimal conditions frequently leads to the initial production of antheridia and can prevent formation of viable archegonia (Page, 1979). Thus, it is possible that the gametophytic ontogenies in the culture experiments reported here do not represent gametophytic ontogenies as realized in nature.

Other factors which undoubtedly have a significant influence are population size, spore output per plant, the influence of the specific aquatic habitat, and the genetic system. It is of interest to note that the highest levels of genetic load were found in the larger populations, suggesting that the frequency and success of recombinants increases with number of individuals as well as age of the population. As spore production by each individual of *A. aureum* is massive, it is probable that inbreeding (in this case, intergametophytic selfing) will occur until such time as there is sufficient spore intermixing to increase the likelihood of outbreeding.

The influence of the aquatic habitat may play an important role in the selection of specific genotypes, perhaps perpetuated by intragametophytic selfing. It is significant to note that work to date on other aquatic species, including *Acrostichum danaeifolium, Ceratopteris thalictroides* and *C. pteridoides,* has provided highly similar results. These species are all characterized by a gametophyte ontogeny which favors intergametophytic mating (including an antheridogen in *Ceratopteris* spp.), but the vast majority of individuals tested express little or no heterozygosity in the form of genetic load. In this regard, Baker (1965) cites seashores and the margins of salt marshes as open habitats where species which are inbreeding with "general purpose genotypes" may be advantageous. Angiosperms which occupy these open and disturbed types of habitats are generally found to be autogamous or apomictic and so are unable to build up recombinants in the population rapidly.

Lastly, the genetic system of pteridophytes must be considered. We still have little understanding of the polyploid system and the maintenance and expression of heterozygosity in these organisms. It is possible that most of them are highly heterozygous and that genetic load is effectively screened from expression. If so, our current methodology for analysis for heterozygosity is insufficient.

It is obvious from these studies that we have little understanding of fern mating systems as they operate in nature and much further work, especially that oriented

toward the genetic system and natural populations of gametophytes and sporophytes, is required before we will be able to circumscribe adequately these phenomena as they operate in nature.

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